Research paper

Mastitis associated transcriptomic disruptions in cattle

Manuela Rinaldi [a,*], Robert W. Li [b], Anthony V. Capuco [b]

[a] *Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium*
[b] *Bovine Functional Genomics Laboratory, Animal and Natural Resources Institute, USDA-ARS, Beltsville, MD 20705, USA*

ARTICLE INFO

ABSTRACT

Mastitis is ranked as the top disease for dairy cattle based on traditional cost analysis. Greater than 100 organisms from a broad phylogenetic spectrum are able to cause bovine mastitis. Transcriptomic characterization facilitates our understanding of host–pathogen relations and provides mechanistic insight into host resistance to mastitis. In this review, we discuss effector mechanisms and transcriptomic changes within the mammary gland in response to experimental infections. We compare temporal, spatial and pathogen-specific local transcriptomic disruptions in the mammary gland as well as pathogen-induced systemic responses and transcriptional changes in distant organs. We attempt to explain why studies on transcriptomic changes during critical physiological periods and in response to non-mastitic pathogens may have important implications for mastitis studies. Future perspectives on revealing bidirectional molecular cross-talk between mastitis pathogens and host cells using cutting-edge genomic technologies are also discussed.

1. Introduction

Mastitis has been ranked as the top disease for dairy cattle based on traditional cost analysis (Wells et al., 1998). Bovine mastitis is defined as an inflammatory condition of the mammary gland in response to injury, which serves to destroy and neutralize infectious agents and promote healing and the return to normal function (National Mastitis Council, 1996). One of the most important factors influencing the outcome of intramammary infections (IMI) is pathogen species and associated virulence factors (Harmon, 1994; Pearson and Mackie, 1979). At least 137 microorganisms from a broad phylogenetic spectrum, including bacteria, yeast, fungi and algae, are able to cause bovine mastitis (Watts, 1988). However, only 5 species of bacteria account for the bulk of bovine mastitis cases. Interestingly, dominant causal agents for mastitis may bear some geographical signatures, as the distribution of

mastitis-causing bacteria display substantial geographic variation (Olde Riekerink et al., 2008).

Gram-negative coliform bacteria, such as *Escherichia coli*, often cause heavy, acute inflammatory responses in the bovine mammary gland and severe clinical symptoms. In contrast, infections by other pathogens, such as Gram-positive *Staphylococcus aureus* or *Streptococcus uberis*, display mild or less severe clinical symptoms, which are often subclinical in nature (Bannerman et al., 2004b; Smith and Hogan, 1993). Rapid recognition of pathogens by host cells is of critical importance in eliciting a rapid innate immune response, a key determinant of the outcome of an infection (Bannerman, 2009). The immune response associated with mastitis is a very complex biological process, involving not only resident and recruited immune cells, but also mammary epithelial and endothelial cells. Both acute and chronic mastitis result in a dramatic increase in somatic cell count (SCC) in milk, with neutrophils being the predominant leukocyte found in both infected mammary quarters and milk (Burton and Erskine, 2003; Paape et al., 1979; Rainard and Riollet, 2003). Distinctive differences in the innate immune response, measured as variations of sol-

* Corresponding author. Tel.: +32 9 264 7815; fax: +32 9 264 7496.
E-mail address: Manuela.rinaldi@UGent.be (M. Rinaldi).

uble inflammatory mediators in milk, have been reported after intramammary injection of Gram-positive and Gram-negative bacteria into the mammary gland (Bannerman et al., 2004b). These studies demonstrate the importance of dissecting the immune response of the mammary gland to different etiological agents, using transcriptomic profiling to understand the patho-physiology of mastitis and to develop appropriate control strategies.

High-throughput genomic technologies, such as high-density microarrays and sequencing-based tools [serial analysis of gene expression (SAGE) and massively parallel mRNA sequencing (mRNA-seq)], provide whole genome approaches to address biological questions. These technologies enable transcriptomic profiling by simultaneously measuring the quantity of transcripts for thousands of genes (Schena et al., 1995) and have become an indispensible part of a biologist's toolbox. In recent years, the application potential of microarray technology has been extended from expression profiling to identifying candidate genes or causative mutation underlying quantitative trait loci (QTL) to aiding genome annotation. The technology has been used to examine the complex interaction between the host and bacterial pathogen (Zheng et al., 2006). Detection of concurrent temporal expression patterns in pathogens and host cells provides an important means to define the pathogenesis of an infection (Meier-Trummer et al., 2009).

The transcriptome is dynamic and responsive. Numerous biological and physical entities, as well as temporal and environmental factors, are able to alter the transcriptome signature. Capturing subtle transcriptomic changes can provide insight into molecular mechanisms underlying biological processes. In addition, microarrays provide a rapid and cost-effective means for detection of bacterial (Cremonesi et al., 2009) and viral (Jack et al., 2009) pathogens. Pathogen microarrays have been used to define pan-genome structures of *S. uberis* (Lang et al., 2009) and *Mycobacterium avium, subsp. paratuberculosis* (Castellanos et al., 2009), which will facilitate the development of efficacious vaccines. In the past 10 years, microarray-based transcriptomic profiling has been used extensively in mastitis studies. In this review, we attempt to highlight findings from transcriptomic profiling of cells and tissues during IMI. We summarize genes, pathways and regulatory networks that are impacted by IMI, and we discuss use of such transcriptomic data to generate novel hypotheses for future work. We also briefly discuss some emerging technologies and use of combined or integrated analysis of genome-scale datasets from various disciplines to increase understanding of the implications of physiological and pathological outcomes of mastitis.

2. Effector mechanisms and transcriptomic changes in milk somatic cells

Early detection of mastitis and identification of associated causative agents allow a timely selection of proper treatment schemes. Therefore, developing simple, reliable and effective biomarkers is of critical importance for the well being of cows. Studies using proteomic and genomic technologies to understand expression of genes in milk

somatic cells and their regulation are at least partially driven by the desire to identify novel biomarkers. Milk cellular defense and soluble effector molecules, such as cytokines, acute phase proteins (APP) and complement factors, represent important components of innate immunity in the mammary gland. The importance of cytokines during the course of IMI and the patterns of expression in milk that are elicited by different mastitis pathogens have been well characterized (Bannerman, 2009). Increased concentrations of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-8, IL-12, interferon (IFN)-γ and tumor necrosis factor (TNF)-α in milk have been well documented responses to IMI. A greater magnitude of expression of these cytokines in milk is often observed in IMI induced by Gram-negative bacteria than by Gram-positive bacteria or mycoplasma, which tend to induce a weaker and more delayed response (Bannerman et al., 2004b; Kauf et al., 2007; Rambeaud et al., 2006). Resolution of the inflammatory response is mediated by up-regulation of inflammatory regulators, such as IL-10, in IMI caused by *E. coli* and *S. uberis*. However, *S. aureus* fails to induce this response in the host (Bannerman et al., 2004b). An early defense mechanism against infection also includes molecules that are related to the acute phase response. Some of the most important of these molecules in milk, produced at a similar level in IMI caused by *E. coli*, *S. aureus*, or *S. uberis*, are haptoglobin (HP), serum amyloid A (SAA) and lipopolysachcharide (LPS) binding protein (LBP) (Bannerman et al., 2004a,b). In addition, activation of complement plays an important role in mammary defense. Increased levels of C3 and C5a complement components in milk, which are known to play important roles in leukocyte recruitment to the mammary gland and stimulation of neutrophil bactericidal activity, are observed in cases of mastitis caused by *E. coli* and *S. uberis*; whereas C5a is usually not detected in milk after intramammary inoculation with *S. aureus*, even though neutrophils are recruited to the gland (Bannerman et al., 2004a,b; Rainard and Riollet, 2006; Shuster et al., 1997).

Milk somatic cells (MSC) represent an important cellular component in the innate immune defense of the mammary gland. Under normal conditions, macrophages represent the predominant cell type (54–83%) in milk, followed by lymphocytes (10–27%) and neutrophils (0–11%). During mastitis, neutrophils become the predominant (>95%) cell type in milk (Lee et al., 1980; Sordillo et al., 1997). During the acute phase of mastitis, MSC increase rapidly and can reach 10^6 cells/ml or greater. Transcriptome analysis of isolated MSC reveals differences in gene expression in infected versus control quarters, as well as among MSC isolated during infections caused by different pathogens (Boulanger et al., 2003; Lee et al., 2006; Pisoni et al., 2010). MSC from normal healthy goats express genes that are primarily related to transcription regulation (12%), signal transduction (8.1%), RNA processing (7.4%) and metabolism (7.3%), while expression of genes involved in immune response represent 1% of the total expressed genes (Pisoni et al., 2010). A significant transcriptomic disruption in MSC becomes evident 24 h post experimental *S. aureus IMI*. The most readily inducible classes of genes in goat MSC include pro-inflammatory cytokines, chemokines and their

receptors; IL-1α, lymphotoxin α, granulocyte chemotactic protein (CXCL6), and IL-2 receptor γ are all up-regulated in infected glands versus healthy controls. In cattle, MSC isolated from uninfected quarters express only regulatory cytokines, such as IL-4, IL-10, and IFN-γ while during infection with *Streptococcus* spp., both regulatory and pro-inflammatory cytokines (IL-1α, IL-8, TNFα) are expressed (Peli et al., 2003).

Several published studies provide further evidence for important roles played by MSC in the activation and the perpetuation of an immune response in the mammary gland during infection. MSC isolated from quarters infected with *E. coli* and *S. aureus* showed increased levels of mRNA for pro-inflammatory cytokines, e.g. IL-6, IL-8, IL-12, GM-CSF and TNFα, compared to those from control quarters; but the magnitude and rapidity of gene response was greater during *E. coli* than *S. aureus* infection (Lee et al., 2006). In MSC from cows infected with *S. aureus*, pro-inflammatory cytokines, chemokines and their receptors were significantly induced (Tao and Mallard, 2007). For example, chemokines, CCR8 and RANTES, and cytokines, IL-17 and M-CSF, antigen presenting molecules, MHC1 and TAP2, and pattern recognition molecules, CD14 and ICAM3, were up-regulated in MSC from infected quarters compared to control. These observations indicate that after recognition of invading pathogens, MSC are able to initiate a signal to recruit cellular factors of immune defense from the blood to the mammary gland. This is consistent with the findings (Alluwaimi et al., 2003) that MSC isolated from quarters infected with *S. aureus* displayed a marked increase in mRNA expression for an array of cytokines (IL-6, IL-12, GM-CSF and TNF-α) involved in the stimulation of the inflammatory response. Interestingly, in the same study, IFN-γ and IL-2 mRNA levels continuously declined as the infection progressed. The ability of *S. aureus* to suppress these two cytokines may be related to the reported capacity of this pathogen to induce a shift in the population of T cells from cells expressing CD4+ markers to those expressing CD8+ (Park et al., 1993; Riollet et al., 2000a). Expression of 2 key enzymes in leukotriene and prostaglandin biosynthesis, 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2), has been evaluated and compared to MSC isolated from control cows with low and high SCC in at least one quarter (Pfaffl et al., 2003). Both genes were up-regulated in MSC from quarters with high SCC. Prostaglandin and leukotrienes are important inflammatory mediators involved in increasing vascular permeability, chemotaxis and hyperalgesia. These findings provide further evidence that MSC play important roles in the activation and maintenance of immune responses in mammary tissue and in pathogen-laden milk.

3. Mastitis associated local transcriptomic disruptions

3.1. Microarray platforms and their application in mastitis studies

3.1.1. Bovine microarray platforms

Since its advent in the early 1990s (Schena et al., 1995), microarray technology has been extensively applied to biological research. Ten years after the first published report, PubMed citations of DNA microarrays reached 5000; and the number has been rapidly growing. Dozens of microarray platforms have since been developed for cattle and deposited in the NCBI Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/). The 10 platforms that are associated with most of the experimental samples deposited in GEO as of March 2010 are listed in Table 1. As indicated, several platforms have been utilized in mastitis studies. These studies range from understanding diverse functions of various mammary tissues (Rinaldi et al., 2010) or cell types (Swanson et al., 2009) in the mammary gland to contrasting differential transcriptomic changes induced by various bacterial pathogens (Jaffrezic et al., 2007).

3.1.2. Pathogen arrays

While many attempts have been made to understand pathogen-induced transcriptomic changes in host cells or tissues, a universal array, based on 16s rDNA sequences, has been developed recently for the detection of mastitis pathogens (Cremonesi et al., 2009). This platform, coupled with a highly discriminative DNA ligation detection reaction, allows detection of 15 pathogens, including both common and emerging pathogens that cause bovine mastitis, such as *S. uberis, S. aureus* and non-aureus staphylococci, as well as *Streptococcus canis* and *Mycoplasmas*. The detection is sensitive (as low as 6 fmol) and specific, having good concordance with standard microbiological analysis. This technology enables a rapid, sensitive, and reliable identification of major mastitis pathogens directly from milk samples, and should facilitate our understanding of host–pathogen relations in cattle. Furthermore, it should be applicable to frozen (archived) tissue and milk samples, thus permitting retrospective studies. The pragmatic implication of this low cost diagnostic tool will be to facilitate the development of early and efficacious intervention strategies.

S. uberis is generally considered a pathogen of environmental origin. However, recent evidence suggests that a few potentially host-adapted ecotypes may exist; and contagious transmission mode for *S. uberis* mastitis is possible (Schmitt-Van de Leemput and Zadoks, 2007; Tomita et al., 2008). A whole genome array based on a published genome, *S. uberis* 0140J, has been developed (Lang et al., 2009). This array, consisting of 1855 of the 1870 open reading frames (ORF) of the *S. uberis* 0140J genome, has been used to define the core genome structure of this bacterium by comparing genes of 21 *S. uberis* strains representing a broad geographical distribution. The core genome, genes common to all strains of *S. uberis,* includes 1530 ORFs; while its dispensable genome consists of genes related to protection responses, small molecule degradation, and two-component regulatory systems, all of which may define niche utilization and adaptation. Such efforts will undoubtedly facilitate our understanding of the pangenome structure and its evolution, and facilitate design of vaccines that are efficacious for a broad spectrum of isolates.

Similar approaches have been utilized to study *S. aureus* pathobiology. Methicillin-resistant *S. aureus* ST398 isolates

Table 1
Bovine microarray platforms deposited in the Gene Expression Omnibus (GEO) database.

Platform	Sample	Transcript	Technology	Description
GPL8776*	641	13,257	Oligo (spotted)	UIUC *Bos taurus* 13.2K 70-mer oligoarray
GPL2112*	436	24,128	Oligo (*in situ*)	Affymetrix Bovine Genome Array
GPL8564	191	8360	Oligo (spotted)	MSU *Bos taurus* BLO 8400
GPL3301*	174	46,490	Oligo (*in situ*)	USDA Bovine 60mer 344K array
GPL4197	147	21,168	cDNA (spotted)	Bovine muscle and adipose cDNA array
GPL6284	96	1920	cDNA (spotted)	INRA-BDR Bovine D14 Embryo 1K
GPL5751	68	3888	cDNA (spotted)	Bovine Total Leukocyte cDNA microarray
GPL5172	56	1680	cDNA (spotted)	UofG_Mallard_Bovine_Immune-endocrine
GPL9712	48	21,536	Oligo (*in situ*)	Agilent-015354 Bovine Oligo Microarray
GPL6497*	36	20,736	cDNA (spotted)	AgResearch *Bos taurus* clone array

Note: (1) Samples from different versions of the same platform are combined and the numbers only represent those deposited in GEO as of March 2010. Only 10 bovine microarray platforms with the most experimental samples submitted to GEO are listed. (2) Transcript contents are from the platform report and may contain limited transcripts for the purpose of quality control and unrelated to the bovine transcriptome. Transcripts could be either annotated genes in the bovine RefSeq database or unassembled ESTs. (3) Asterisks (*) denotes platforms used in studies related to the bovine mammary gland and mastitis.

have been characterized using molecular tools including diagnostic DNA arrays for genetic relatedness, antimicrobial resistance and virulence properties (Fessler et al., 2010). A uniform virulence gene pattern is found to be conserved between ST398 isolates from both swine and cattle.

3.2. Impact of transcriptional changes during phases of mammary development and function on mastitis

Susceptibility of the mammary gland to new infections is markedly increased during critical physiological transitions such as the early dry period (Nickerson, 1989) and the peri-parturient period (Smith et al., 1985). These periods are characterized by marked changes in nutritional demand and mammary secretory activity, cell turnover and tissue remodeling. Understanding molecular events during mammary development and these transition periods should provide mechanistic insight into physiological changes that render the mammary gland more susceptible to mastitis and help develop better prevention and intervention strategies.

The mammary gland undergoes repeated cycles of growth, differentiation and regression in accordance with the reproductive strategy of the mammal. Accordingly, the mammary gland undergoes a series of dramatic phenotypic changes during pre-pubertal, gestational and lactational stages. In cows, there is rapid ductal growth and considerable branching prior to puberty (Capuco et al., 2002). Although some additional growth occurs during repeated estrous cycles, the majority of mammary growth and differentiation occurs during pregnancy. During pregnancy there is extensive ductal branching and formation of mammary alveoli, culminating in the cytological and biochemical differentiation of the mammary epithelium necessary for onset of lactation (Capuco et al., 2002). With the cessation of milk removal, there is extensive mammary cell turnover and tissue remodeling in all species, but the extent of involution is greatly influenced by the pregnancy status of the animal (Capuco and Akers, 1999).

Transcriptomic analysis has been extensively utilized to evaluate gene expression during various phases of mammary gland development in rodents (Blanchard et al., 2007;

Clarkson and Watson, 2003; Clarkson et al., 2004; Lemay et al., 2007; Ron et al., 2007; Stein et al., 2004). These studies show that dominant expression patterns or pathways are associated with each of the important stages in murine mammary development. Enhanced expression of genes related to milk protein biosynthesis and protein transport is evident during lactation, consistent with the requirement for large-scale production of milk proteins (Master et al., 2002). Apoptosis of alveolar epithelial cells and tissue remodeling are hallmarks of changes that occur in response to milk stasis. In response to forced weaning (∼at peak lactation) in rodents there is extensive apoptotic cell death and involution. Therefore, it is not surprising that this involution is characterized by a transient increase in expression of death receptor, increase of apoptosis, inflammatory cytokines and acute phase response genes (Clarkson and Watson, 2003; Clarkson et al., 2004). Although much can be learned from rodent studies, it is important to appreciate potential shortcomings of mice as models for bovine mammary gland biology. An appreciation of differences between mice and cattle with regard to tissue architecture (which can influence cell–cell interactions), endocrine regulation and other physiological aspects is important.

Particularly pertinent to this review are concerns about the applicability of mammary involution studies in mice to physiology of the bovine mammary gland during the dry period (Capuco and Akers, 1999). Cessation of milking in dairy cows typically occurs after a prolonged lactation and during the final months of pregnancy, whereas mammary involution has typically been studied in non-pregnant mice following forced weaning close to peak lactation. Thus, when milk stasis occurs in dairy cows (dry period), the mammogenic and lactogenic stimulation of pregnancy opposes stimuli for mammary involution. The descriptive term "regenerative involution" has been used to describe tissue remodeling during the dry period, characterized by extensive cell turnover with little or minimal cell loss, and to distinguish it from the extensive involution process studied in mice (Capuco et al., 2003). Even in the absence of pregnancy, involution after cessation of milk removal is slower and less pronounced in cows than in mice (Wilde et al., 1999).

Molecular events occurring following milk stasis should therefore be examined in the bovine mammary gland. Although not evaluated during a typical dry period, a recent study showed that the genes with greatest changes in the bovine mammary gland following cessation of milking were related to oxidative stress, immunity and cell survival (Singh et al., 2008). At least 7 genes related to oxidative stress, such as superoxide dismutase 2 (SOD2) and metallothionein 1A (MT1A) were up-regulated at 36 h post-milking. The majority of genes encoding for milk proteins were down-regulated during involution, which, along with decreased expression of cell survival signaling, may represent a protective response to oxidative stress that occurs prior to epithelial cell apoptosis and induction of immune responses in the bovine mammary glands (Singh et al., 2008). Analysis of the mammary transcriptome during a typical dry period has yet to be reported, but is currently in progress (Capuco et al., 2002, 2003, 1992, 2001).

The early dry period and late dry period represents critical physiological stages of compromised immune status. High incidence of severe clinical mastitis is often observed during these periods (Erskine et al., 1988), especially for *E. coli* mastitis (Burvenich et al., 2007). The increased incidence is related to a decreased function of the immune system (Burton and Erskine, 2003), particularly, a decreased functionality of neutrophils (Lamote et al., 2006), including impaired chemotaxis, superoxide production and phagocytic capabilities (Cai et al., 1994; Lee and Kehrli, 1998). Changes in antioxidant defense mechanisms and proinflammatory markers within bovine mammary tissue during the final month of the dry period and transition to lactation were consistent with increased oxidative stress and susceptibility to mastitis during this period (Aitken et al., 2009).

During the dry period, changes in endocrine and local factors may impact immune function (Burvenich et al., 2007; Pezeshki et al., 2010). Circulating estrogens, which increase during late pregnancy, have been suggested to serve as immunomodulators, reducing the efficacy of the immune response. For example, estrogen treatment of bovine blood neutrophils significantly decreases the expression of CD47 (Lamote et al., 2004), a molecule involved in the migration of bovine neutrophils through collagen, reduces the number of viable neutrophils, which could alter the inflammatory response (Lamote et al., 2004), and is believed to decrease neutrophil function (Burvenich et al., 2007). In an effort to study the impact of estrogens on prepubertal growth of mammary ducts, we demonstrated that approximately 2344 genes responded significantly to administration of estradiol, with differential responses among cell types (Li et al., 2006; Li and Capuco, 2008). Some of the genes influenced by estrogen status are known to be immunomodulators. Thus, effects of estrogens on immune functions may be indirect, mediated by cells other than the target cell and mediated by local factors. The same is undoubtedly the case for other hormones. Progesterone at high concentration reduces neutrophil oxidative burst activity (Moreira da Silva et al., 1997) and lymphocyte activation (Wyle and Kent, 1977). Glucocorticoids which promote parturition and respond to stressful stimuli generally suppress immunity and may play a role in the depression of immune function at parturition, despite their ability to induce neutrophilia at lower circulating concentrations (Pezeshki et al., 2010; Burvenich et al., 2007). Prolactin receptor is expressed on most immune cells and prolactin is secreted by lymphocytes, suggesting a role for this hormone in endocrine or paracrine modulation of immune function, particularly since both hyperprolactinemia and hypoprolactinemia are immunosuppressive (Matera, 1996). Because there is a cessation of milking-induced release of prolactin at dry-off and a surge in prolactin secretion periparturiently, this hormone may be implicated in altered immunity during these critical transition periods.

In addition to the altered hormonal and paracrine milieu of mammary tissue during the dry period, other factors clearly play a role in altering immune function during this transition period. Milk stasis during the early and late (periparturient) dry period undoubtedly influences the bactericidal capacity of neutrophils. Ingestion of milk components, most notably milk fat globules, impairs the phagocytic capability of neutrophils recruited to the mammary gland (Paape et al., 1979). This impairment is compounded by the lack of milking-induced turnover of neutrophils. Notably, cows typically experience a period of negative energy balance (NEB) periparturiently due to increased energy demand for fetal growth and milk production and reduced feed intake (Ingvartsen and Andersen, 2000). NEB in mid-lactation Holstein cows alters the expression of genes involved in immune responses to a *S. uberis* intramammary challenge (Moyes et al., 2010a,b). NEB down-regulates genes associated with antigen presentation, respiratory burst and cytokine secretion, but up-regulates expression of genes involved in TLR signaling (TLR2 and TLR4) and cytokine secretion (IL-6). The repressed expression of HLA-DRA, a gene encoding for MHC II, suggests that NEB likely contributes to the impaired expression of MHC II in PMN during the peri-parturient period. Altered expression profiles of blood PMN in cows during NEB and the resultant impaired immune responses may be responsible for increased susceptibility of cows to mastitis during this critical physiological period. Others have demonstrated that alterations in blood metabolites (low glucose and elevated ketone bodies) associated with NEB decrease neutrophil phagocytic and killing activity (Hammon et al., 2006; Burvenich et al., 2007; Grinberg et al., 2008). Transcriptome profiling of spleen from cows with severe negative energy balance during early lactation showed changes in gene expression consistent with a negative impact on innate and adaptive immunity (Morris et al., 2009). Affected pathways were associated with NRF2-mediated oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, natural killer cell signaling, p53 signaling, down-regulation of IL-15, BCL-2, and IFN-γ; up-regulation of BAX and CHOP and increased apoptosis. In the liver feed restriction and ketosis resulted in severe down-regulation of genes involved in protein synthesis, protein trafficking, protein ubiquitination, and molecular transport, i.e. the liver responded to NEB by shutting down energy-generating processes (Loor et al., 2007). These responses promote compromised immune function.

3.3. Shifts in pathways and regulatory networks during mastitis

3.3.1. Innate immune responses common to all infections

Transcriptome profiling has enabled the identification of genes, pathways and regulatory networks activated in mammary tissues during experimental infection by various pathogens, including *E. coli, S. aureus* and *S. uberis* (Gunther et al., 2009; Lutzow et al., 2008; Moyes et al., 2009; Rinaldi et al., 2010; Swanson et al., 2009). These studies demonstrate that in the first few hours following infection, the mammary gland undergoes a marked transcriptomic disruption. For example, immune and inflammatory responses, cell proliferation, apoptosis and remodeling of extracellular matrix are among the pathways commonly activated by diverse pathogens (Gunther et al., 2009; Moyes et al., 2009; Rinaldi et al., 2010). Lipid metabolism in the mammary gland is generally inhibited by infection (Rinaldi et al., 2010). Increased expression of TLR2 and TLR4 is observed in the mammary gland sampled within a few hours of infection, seemingly independent of pathogens (Lutzow et al., 2008; Moyes et al., 2009; Rinaldi et al., 2010). In *E. coli*-induced mastitis, TLR2 and TLR4 share a similar tissue pattern with maximum expression in the teat (Rinaldi et al., 2010), including Fürstenburg's rosette (FR) and teat cistern (TC). During IMI by *S. aureus* and *S. uberis,* both TLR2 and TLR4 are found to be up-regulated (Lutzow et al., 2008; Moyes et al., 2009), even though LPS, the ligand for TLR4, is generally absent in these bacteria. These findings are consistent with a published report that increased levels of TLR2 and TLR4 mRNA molecules are observed in udders with natural infections caused by various pathogens (Goldammer et al., 2004). Induction of these TLRs is not a systemic phenomenon but is confined to the infected quarter of the udder. These results demonstrate that rapid pathogen recognition by TLRs is evident in all regions of the mammary gland. Interestingly once recognized by the TLRs, the downstream signaling pathways start to diverge between pathogens. For example, *S. aureus* does not activate NF-κB in mammary epithelial cells, likely causing a diminished immune response (Yang et al., 2008). This finding may partially explain why *S. aureus* infection is usually characterized by a weak inflammatory response *in vivo* compared to coliform bacteria (Bannerman et al., 2004b; Riollet et al., 2000b). Pathways related to certain innate immune responses, such as leukocyte chemotaxis and neutrophil activation are strongly up-regulated by IMI independently from the pathogen causing infection. For instance, expression of CCL2, CCL4, CCL20, CXCL2 and IL8, essential proteins for the recruitment of leukocyte into the mammary gland, are up-regulated during *E. coli* and *S. aureus* infection in the mammary gland at 12 and 16 h, respectively (Lutzow et al., 2008; Rinaldi et al., 2010). Numerous pro-inflammatory cytokines involved in the acute phase response, like IL-1β, IL-6 and TNF are also significantly up-regulated in mammary tissues during infection (Lutzow et al., 2008; Rinaldi et al., 2010). IL-10 and IL-6 signaling pathways are activated during *S. uberis* experimental challenge (Moyes et al., 2009).

Commensal bacteria such as *Bacillus subtilis* and *Bacillus cereus* have been marketed as gastrointestinal probiotics because of their inhibitory properties against Gram-positive bacteria, including potential mastitis pathogens (Hoa et al., 2000). Certain species of lactobacilli, such as *Lactococcus lactis*, a common food component, have been shown to reduce staphylococcal counts in human milk without eliciting any clinical mastitis symptoms and provide protection against a few mastitis pathogens. Interestingly, this non-pathogenic bacterium is also able to elicit a strong up-regulation of TLR2 and TLR4 as well as proinflammatory cytokines, such as IL-1β, IL-8 and TNFα (Beecher et al., 2009). The expression of CXCR1, IL-10 and IL-12 is also induced. Clearly, this rapid innate immune response is not driven by pathogen virulence factors.

3.3.2. Temporal shifts in biological pathways during intramammary infection

Upon entering the host, bacterial pathogens rapidly proliferate. The interaction of bacterial pathogens with the host is intimate and bidirectional, and determines infection outcome. In *E. coli*-induced mastitis, studies show that severity of infection is mainly determined by host factors rather than bacterial pathogenicity (Burton and Erskine, 2003). On the other hand, pathogenic virulence factors play a critical role in other host–pathogen systems. When mechanisms of molecular cross-talk between pathogens and host cells in bovine mastitis remain largely unclear, host transcriptomic responses are very rapid and become magnified and widespread as infection progresses (Rinaldi et al., 2010). At individual gene levels, several studies have monitored the temporal changes of selected cytokines (Bannerman et al., 2004b; Lee et al., 2006). The temporal profile of IL-12 mRNA levels following infections by *E. coli* and *S. aureus* is similar, with a rapid increase up to 16 h post infection (p.i.) and peaking at 24 h p.i. (Lee et al., 2006). The expression of IL-8 reaches a plateau at 16 h post *E. coli* infection and then gradually returns to basal levels in MSC (Lee et al., 2006). In the secretory or lobulo-alveolar (LA) region of the bovine mammary gland, 576 genes were significantly impacted 2-fold or greater ($P < 0.05$) by *E. coli* at 12 h p.i. (Rinaldi et al., 2010). When the infection progressed to 24 h, 4243 genes, approximately 10% of the all transcripts tested, were significantly affected, coinciding with maximum biological and clinical symptoms. The transcriptomic response at this scale is striking but not unexpected. Pathway analysis identified 47 canonical pathways significantly ($P < 0.05$) impacted at 12 h p.i., while 94 pathways were significantly regulated at 24 h p.i. Thirty six pathways, mainly signaling pathways, were activated at both time points, including the acute phase response and integrin signaling, IL-4, IL-6, IL-9, IL-10, IL-17 and IL-22 signaling pathways, NF-κB and P38 signaling pathways, PPAR signaling and FXR/RXR as well as LXR/RXR activation pathways. Many pathways, including 3 pathways shown in Fig. 1, displayed significant responses at only one time point. Leukocyte extravasation signaling was only affected at 24 h p.i., suggesting that a significant leukocyte recruitment and infiltration may be mobilized in the LA region at this time point. At 12 h p.i., only one gene, CD55, in the complement cascade was up-regulated with no significant activation of complement. When the infection progressed to 24 h, at least 9 genes in the 36-gene complement pathway were significantly affected in the LA
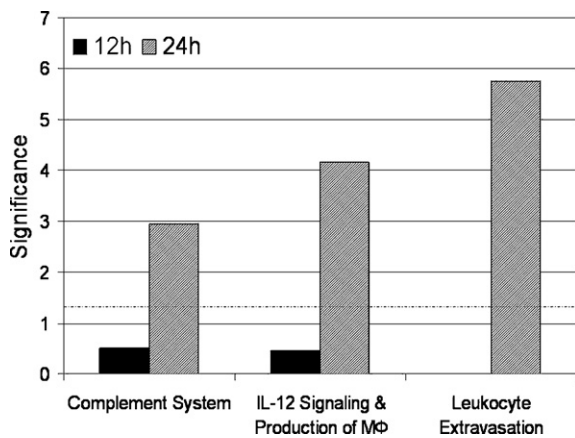
Fig. 1. Pathways impacted in the lobulo-alveoli region of the bovine mammary gland during *E. coli* infection based upon the level of significance. The dashed line represents a significance level at $P < 0.05$. The normalized microarray data were downloaded from the GEO database (accession # GSE15441) and analyzed using *t*-test. The significant genes were sorted based on *P*-value ($P < 0.05$) followed by fold change (≥ 2.0). The genes with annotation were subject to pathway analysis using the Ingenuity Pathways Analysis (IPA v8.5).

region. Significant activation of the complement cascade at 24 h p.i. suggests that local complement activation may play a role in eliminating bacterial pathogens from the host.

3.3.3. Pathogen-specific transcriptomic changes

Bacterial effector molecules, such as pathogen virulence factors and protein secretion apparatus found in both animal and plant pathogenic bacteria, dictate biochemical cross-talk between bacteria and host cells, which in turn dictates disease progression (Salmond and Reeves, 1993). While certain aspects of innate immune responses are conserved and represent a common immune response to pathogenic bacteria, different pathogens induce specific transcriptomic responses. Understanding the uniqueness of pathogen-specific responses will undoubtedly facilitate vaccine design and the development of effective intervention strategies. It has long been known that *E. coli* mastitis tends to be acute and of short duration, whereas *S. aureus* mastitis is often chronic and persistent (Smith and Hogan, 1993; Sutra and Poutrel, 1994). The difference in innate immune responses to different pathogens is often obvious. For instance, there is up-regulation of IFN-γ mRNA in MSC isolated during *E. coli* mastitis, but not during *S. aureus* mastitis (Lee et al., 2006). Typically, intramammary injection of *E. coli* induces a faster and more robust cytokine response than does comparable infection with *S. aureus* (Bannerman, 2009). The compromised up-regulation of inflammatory cytokines in *S. aureus*-infected glands may partially contribute to the chronic nature of this infection (Lee et al., 2006). Pathogen-specific responses can also be detected between Gram-negative bacteria, e.g. *E. coli* and *Psuedomonas aeruginosa*. Elevated levels of soluble CD14, a key molecule mediating LPS and TLR4 interaction, are sustained for a longer period following *P. aeruginosa* infection in milk (Bannerman et al., 2005). Whole transcriptomic responses induced by different pathogens have naturally attracted much attention. The number of differ-

entially expressed genes was reported to reach a maximum of ~1190 genes 24 h post *E. coli* infection in bovine mammary gland (Jaffrezic et al., 2007), in an agreement with our data in the LA region of the gland (Rinaldi et al., 2010). However, not a single gene was significantly regulated during *S. aureus* infection at the same time point (24 h p.i.), under the same analysis stringency and cutoff value (false discovery rate or FDR < 0.05). When using a relaxed cutoff criterion ($P < 0.01$ and fold change at 1.5 or greater), 266 genes were impacted 24 h post *S. aureus* infection. Biological and sampling variations alone may not account for the disparity. Instead, differences in underlying molecular mechanisms may be involved. Leucine-rich repeat kinase 2 is down-regulated at all 4 time points tested during *S. aureus* but not *E. coli* infections (Jaffrezic et al., 2007). Similarly, comparison of transcriptomic responses of the bovine mammary gland to *E. coli* and *S. uberis* IMI has recently been made available (http://milkgenomics.org/groups/Bovine_Lactation_CP/microarray-data/).

3.3.4. Cellular response depends on region within the gland, time after infection and pathogen

Although the streak (teat) canal provides an important physical and chemical barrier to bacterial entry into the mammary gland (Capuco et al., 1992), little is known about the local response of tissues within the teat or other regions of the mammary gland to bacterial infection. Understanding the spatial and cellular expression patterns of bactericidal effector molecules, as well as the regulation of initial events controlling immune defense within the udder, may facilitate development of schemes to increase the mammary gland's immune defense against mastitis pathogens. Bacteria invade the mammary gland through the streak canal and the first tissues to encounter invading pathogens are those of the teat. Invasion of pathogens progresses from the teat to the gland cistern (GC) and then to the dorsal extremities of the mammary gland where milk is synthesized within the alveoli. To assess regional responses throughout the gland during *E. coli* infection, we conducted a global evaluation of transcriptional changes in tissues collected from four regions of control and infected mammary glands (Rinaldi et al., 2010). We examined the responses of tissue collected from the Furstenberg's rosette (FR), teat cistern (TC), gland cistern (GC), and lobulo-alveolar (LA) regions 12 and 24 h after IMI with *E. coli*. Widespread changes in gene expression were observed, with time-dependent regional differences in transcriptome profiles. Across all regions, the top networks activated by *E. coli* infection pertained to immune and inflammatory responses and genes involved in extracellular matrix remodeling (e.g. matrix metallopeptidase 1 and 9; ADAM8). Interestingly the teat responded most rapidly and strongly to infection. At 12 h p.i. the number of genes impacted as well as the magnitude of their response was greatest in tissues of the teat (TC and FR), while the LA was the most responsive region at 24 h. The FR is a small anatomical region situated at the internal end of the streak canal. It is worthy of note that inducible nitric oxide synthase (NOS2) a gene involved in the production of bactericidal molecules was strongly up-regulated (96.6-fold) in this region as early as 12 h p.i. The reason for the

observed time dependent difference among regions of the mammary gland is uncertain. However, the experiment was designed to permit injection of a limited number of *E. coli*, with the expectation that the IMI would spread from the site of inoculation in the teat cistern. Data support the concept that the teat provides an important sentinel function and reacts strongly during the early stage of *E. coli* IMI. The contribution of immune and non-immune cells to the up-regulation of the defense mechanisms against *E. coli* is unknown. Somatic cells, during the reported *E. coli* infection, did not peak until 24 h p.i. and a minimal influx of immune cells was evident in the histological sections at 12 h, suggesting that in samples collected before the peak of clinical signs (24 h), resident cells in the gland play a major role initiating the immune response. In a previous study (Nickerson and Pankey, 1983), differences in resident cell population among different regions of healthy and infected mammary gland have been reported, therefore differences in anatomical location as well as in resident and recruited cell population at the time of sampling may contribute to the observed shift in tissue response during infection.

Mammary epithelial cells (MEC) are the milk secreting cells and the most abundant cell type in the lobulo-alveolar region of healthy udders (Capuco et al., 2001; Vanselow et al., 2006). Therefore *in vitro* models have been used to test the response of MEC to mastitis pathogens, with the intent of assessing the role MEC may play during *in vivo* infections (Gunther et al., 2009; Swanson et al., 2009). Microarray analyses of the transcriptome response of bovine MEC exposed to heat-killed *E. coli* showed that genes that were up-regulated 1 h after "infection", were primarily immune factors that are regulated by TLR signaling (CCL5, IL6, IL1, NOS2), as well as an array of chemokines (CCL20, IL8, CCL2, CXCL5 and CXCL2) (Gunther et al., 2009). This indicates that MEC are able to recognize the pathogens, and can likely recruit immune cells into the mammary gland. Further up-regulation of transcripts for genes encoding enzymes involved in eicosanoid synthesis (PDGES, ALOX15B) and down-regulation of an enzyme that promotes prostaglandin degradation (HPGD) suggest that MEC are also a significant source of prostaglandin and anti-inflammatory lipoxins in the udder. In another recent study, mammary gland and isolated bovine MEC were infected with *S. uberis* 233 (Swanson et al., 2009). The results reported show that *S. uberis* was able to cause clinical mastitis, with up-regulation of several genes that are involved in immune and inflammatory responses. But *S. uberis* treatment of MEC *in vitro* did not alter expression of selected proinflammatory cytokines or immune related genes [C3, clusterin, IL1B, IL6, IL8, lingual antimicrobial peptide (LAP), SAA3, S100A12, TLR2]. These studies suggest that MEC are capable of initiating an *in vivo* immune response to invading microbes, but the significance of the MEC response depends upon the invading pathogen (Swanson et al., 2009).

3.4. Complications with other pathological conditions

It has long been recognized that non-mastitic pathogens directly or indirectly influence the incidence and progression of intramammary infection. For example, in herds exposed to bovine viral diarrhea virus (BVDV), a 7% increase in the incidence rate of clinical mastitis was observed (Waage, 2000). BVDV infection caused a marked reduction in number and function of circulating leukocytes (neutrophils and lymphocytes), thus increasing the incidence of secondary infections (Roth et al., 1981). At least 14 viral infections, including those by BVDV and bovine herpesvirus 1, have been associated with increased incidence of bovine mastitis, due to the immunosuppressive properties of these viruses (Barkema et al., 2009; Wellenberg et al., 2002). SAGE-based transcriptomic analysis of bovine aortic endothelial cells exposed to BVDV revealed significant increases in the mRNA for many genes, including P-selectin, tryptophan tRNA synthetase and prostaglandin D2 synthase (Neill et al., 2008). Intriguingly, BVDV-exposed cells displayed an altered response to LPS and dsRNA, possibly via over-expression of TNFα-induced protein 3 and inhibition of NF-κB activation. Altered biochemical pathways or functions induced by BVDV are also evident in bovine kidney cells (Neill and Ridpath, 2003). Tubulins are down-regulated by the infection, indicating reduced functionality or possible dysfunction of cell division or other functions where microtubules play a role. Transcriptomic disruptions induced by BVDV strongly suggest that impaired immune function may lead to increased susceptibility to secondary mastitis infections.

Bovine leukemia virus (BLV) is capable of a lifelong infection in the lymphoid tissue of cattle. While the majority of cattle infected by BLV remain clinically asymptomatic throughout their life spans, BLV particles are numerous in lymphocytes in the mammary glands of BLV-positive cows with subclinical mastitis (Yoshikawa et al., 1997). Histological lesions, possibly caused by the virus, are evident and suggest a close association between BLV and mastitis. Recently, global transcriptional profiles of bovine peripheral blood mononuclear cells induced by BLV have been examined (Li et al., 2009). BLV infection altered the response of mononuclear cells to LPS. Among 33 genes significantly regulated by LPS, 32 were down-regulated in BLV-positive cells compared to BLV-negative cells. Network analysis indicated that CDC25A and transcription factors such as STAT1 and STAT3 may serve as important signaling pathways for the BLV-induced cellular responses.

Epidemiological studies suggest a possible association between Johne's disease and mastitis (Tiwari et al., 2005). *Mycobacterium avium subsp. paratuberculosis* (MAP)-seropositive cows have a higher culling rate due to decreased reproductive efficiency, decreased milk production, or mastitis. Association of MAP infection with reduced mastitis is observed in cattle (Wilson et al., 1993). Rates of new and chronic mastitis infections are significantly lower in cows with subclinical paratuberculosis. However, no detailed studies have been reported to establish a solid link between these two infections.

4. Systemic responses and transcriptional changes in distant organs

The ductal network for each mammary gland is self contained and does not connect with the networks in other glands (quarters) of the udder. Left and right quarters are

also separated by the medial suspensory ligament, which serves to additionally isolate the udder halves. Together, these features prevent free migration of bacteria between the four mammary glands. Therefore, quarters adjacent to an infected quarter within the udder remain bacteriologically negative (Mitterhuemer et al., 2010). However, transmission of bacteria from one quarter to another has been described for contagious pathogens such as *S. aureus* and *Streptococcus agalactiae* (Radostits, 1994).

Pathogen infection of the bovine mammary gland induces not only localized changes in gene expression but also a systematic response and transcriptomic disruption in distant organs. In LPS-induced mastitis, systemic responses during the acute phase of the disease include elevated body temperature, increased heart and respiratory rates and increased MSC counts (Vels et al., 2009). In *E. coli*-induced mastitis, acute phase proteins, HP, SAA and LBP appear in serum in waves. SAA first starts to increase in serum, although slowly, and peaks at 12 h p.i., followed by LBP (peaks at 36 h p.i.) and HP (peaks at 60–68 h p.i.). The patterns in milk are similar to those in serum. The first infection tends to induce a stronger response than observed after re-infection (Suojala et al., 2008). In LPS-induced mastitic cows, HP protein starts to accumulate in blood at 6–9 h p.i., compared to 3 h p.i. in milk from infected quarters (HP levels remain unchanged throughout the experiment in contralateral control quarters), suggesting a much delayed systemic response (Hiss et al., 2004).

Blood mononuclear cells (BMC) from *S. aureus*-infected cows have been compared to those from healthy control cows with similar age, parity and lactation stages (Tao and Mallard, 2007). Among the 22 genes significantly regulated by the infection, 8 chemokine and cytokine related genes, such as IL-18, were up-regulated by infection. These chemokines and cytokines may be responsible for the activation and migration of BMC into the site of infection in the gland. The down-regulated genes are mainly involved in antigen presentation. Cytokine expression patterns induced in BMC by *S. aureus* mastitis are distinct from those in milk somatic cells, as expected.

The liver plays a critical role in the acute phase response observed during bovine mastitis caused by *E. coli* (Jiang et al., 2008). Using a minimally invasive liver biopsy technique, the hepatic response to LPS-induced mastitis was evaluated (Vels et al., 2009). Hepatic expression of IL-1β, IL-6, IL-10, and TNFα as well as acute phase proteins, such as SAA3 and HP, were significantly induced by intramammary injection of LPS. In another study, global changes in hepatic gene expression were evaluated following intramammary injection of LPS into the bovine mammary gland (Jiang et al., 2008). Expression of 4610 genes (~20% of all genes tested) was impacted by LPS-induced mastitis at one or more times post-infection (Jiang et al., 2008). Data clearly demonstrate that the hepatic transcriptome is rapidly responsive to LPS administration in the mammary gland. Two clusters of genes, approximately 107 in total, respond within 3 h post injection. These early response genes include 82 transcripts with a rapid burst in expression (and a quick return to the baseline) related mainly to immune and inflammatory responses, chemotaxis, cyclooxygenase pathway and macrophage differentiation. Maximal impact of LPS on

the hepatic transcriptome was observed 9–12 h p.i. Two clusters of genes displayed the greatest changes, including 1146 transcripts with increased expression levels and 1610 transcripts, mainly in various metabolic processes, with down-regulated expression levels during this period. Most transcripts return to the baseline by 48 h p.i. The hepatic transcriptome data suggest that the acute phase response is triggered by activation of signaling pathways that are involved with common and hepatic-specific transcription factors and pro-inflammatory cytokines. These mediators in turn regulate the expression of genes encoding acute phase proteins, collectins, complement components, chemokines, cell adhesion molecules and key metabolic enzymes (Jiang et al., 2008).

Approximately 359 genes, including metallothioneins and LPS binding protein, were differentially expressed in control quarters from an *E. coli* infection, suggesting that an immune response may have been triggered in the uninfected quarters (Jaffrezic et al., 2007). Indeed, a recent study showed that infection with *E. coli* in the bovine mammary gland provokes two distinct types of response to the pathogen (Mitterhuemer et al., 2010). The first response comprises locally restricted reactions. Genes regulated during this wave had a distinct profile of functions, dominated by biological processes related to immune response and inflammation as well as chemokine/cytokine signaling and diapedesis of leukocytes. The second response occurred in infected as well as in neighboring healthy quarters. This systemic response may serve to impair progression of infections to neighboring mammary quarters. Genes impacted during this response include components of the antigen processing and presenting machinery, cytokines, such as CXCL2 and CXCL14, which have been shown to possess direct antimicrobial properties against *E. coli*, and protein degradation and apoptosis. The extent of the interplay of both local and systemic reactions likely determines the course of infection.

5. Combining transcriptomics and QTL analyses

Desires to breed mastitis resistant cows using marker-assisted selection (MAS) have led to the identification of many segregating quantitative trait loci (QTL) that affect mastitis or milk somatic cell score (SCS). A low level of SCS is often considered an indirect predictor of resistance to mastitis. Milk production and susceptibility to mastitis are generally inversely correlated. Several QTLs affecting susceptibility to mastitis and SCS, or both, have been identified. For example, a significant QTL on *Bos taurus* chromosome (BTA) 6 affects clinical mastitis in Norwegian dairy cattle (Klungland et al., 2001). QTL for both SCS and mastitis have been localized in the same distal end of BTA3 and BTA18. The telomeric region of BTA18 shows a strong linkage for both SCS and mastitis (Xu et al., 2006). Several reported QTL related to mastitis and SCS are located at the proximal end of BTA23. In addition, QTL for mastitis and/or SCS have been reported on BTA 1, 14, 21 and 27 (Schulman et al., 2004). However, these QTL, identified by linkage analysis using sparse microsatellites markers within an interval of tens of cM, typically span multiple megabases (Mb) on physical maps and harbors hundreds of genes, and are not sufficient

for MAS. Linkage disequilibrium analysis or genome wide selection based on thousands of single nucleotide polymorphisms (Raadsma et al., 2008) can be used to fine map QTL regions. Identification of genes and causative mutation underlying QTL helps gain insight into biochemical pathways that contribute to traits and phenotypes, and provide accurate markers for MAS. However, this process has been proven rather challenging and time-consuming. Recently an integrated approach utilizing genome information and whole genome transcriptomic profiling to identify candidate genes for QTL has been developed (Lemon et al., 2002; Ron et al., 2007). Combining whole genome transcriptomic profiling with QTL mapping enables researchers to focus on genes localized within QTL regions, providing a shortcut for the understanding of the genetic organization of traits with relative low heritability, such as mastitis. By utilizing ready availability of detailed microarray-based gene expression data from every major stage of mammary development in mice and comparative genomics tools, a web database for candidate genes for QTL for milk production traits in cattle has been developed (Ron et al., 2007).

MAS based on the confirmed mastitis QTL on the BTA 18 shows a pronounced improvement in milk somatic cell counts (Kühn et al., 2008). The heifers of MAS for the BTA18 QTL showed significant improvement in somatic cell score (equivalent to a reduction of 67,000 somatic cells per ml). mRNA expression in mammary epithelial cells from cows with increased mastitis resistance due to MAS, showed elevated levels of several key genes implicated in innate immune response, including IL-1β, IL-6, IL-8, RANTES, TLR2, TNFα and complement component C3 as well as lactoferrin (Griesbeck-Zilch et al., 2009). Recently, a systematic and semi-automatic analysis pipeline has been proposed and applied to combined analysis of microarray and QTL data in order to identify genes underlying tolerance to *Trypanosoma congolense* infection in cattle (Rennie et al., 2008). This pathway-based approach allows a rapid identification of genes underlying a QTL that are not necessarily differentially expressed but nevertheless influence the expression of their downstream targets in a pathway. Such a data-driven analysis tool can be readily employable to mastitis studies.

6. Future directions

Considerable progress has been made in characterizing transcriptomic responses of the mammary gland to infections. The knowledge has advanced our understanding of host–pathogen interactions involved in bovine mastitis and provides mechanistic insight into disease progression. The majority of current studies on transcriptomic characterization focus on experimental infection, which may not accurately reflect the transcriptomic dynamics during a natural infection. Therefore, future studies should attempt to compare differential transcriptional response of various breeds and genotypes (either susceptible or resistant to mastitis) to natural IMI. Special attention should be given to couple transcriptional profiling with QTL analysis and single nucleotide polymorphism (SNP), in order to further our understanding of the molecular mechanisms of host resistance, to increase our knowledge of the genomic basis for

individual variability of response IMI and to provide accurate markers for MAS. Current technological limitations, such as different microarray platforms, non-standardized analysis tools and statistical cutoffs, large biological variation in out-bred populations commonly used in challenge experiments, small sample size and sampling bias in the mammary gland, make direct comparison of microarray results between results published by various labs difficult, if not impossible. Massively parallel sequencing technologies (mRNA-seq) allow direct counts of mRNA molecules down to a single molecule per cell, rapid detection of splice variants, allele-specific expression, and sequence variation at single nucleotide resolution. Our preliminary results identify at least 16,859 bovine Refseq transcripts (genes) expressed at a level ≥1 molecule in the bovine mammary parenchyma. This technology, with a potential to remove hybridization-related artifacts, could provide a powerful alternative to microarray-based transcriptomic profiling. Infiltrated immune cells in mammary tissue initiate and maintain local innate immune responses in the infected tissue and play a critical role in guiding disease progression. Studies show that the mammary gland and a primary cell culture model display distinctly different transcriptome profiles (Swanson et al., 2009). Different transcriptomic responses of various cell types isolated *in situ* using laser capture microdissection will help to clarify the contribution of various cell populations to the host response. A precise knowledge concerning the cell type expressing bactericidal molecules as well as the structure and regulation of initial events controlling the immune defense in the udder may provide experimental alternatives to enhance protection of the gland against mastitis pathogens. Moreover NEB negatively impacts liver functionality during IMI (Loor et al., 2007), this in turn will impact host response. A better understanding of the nutrient requirements of host cells and pathogens, could lead to new management and treatment strategies that do not require the use of antibiotics or other drugs to prevent and combat mastitis during periods of metabolic stress. The simultaneous detection of temporal expression patterns of pathogens and host cells, especially during *S. aureus* mastitis, will help to define the pathogenesis of mastitis and provides insight into molecular cross-talk between pathogens and host cells. Defining pan-genomic structures of major mastitis pathogens using 2nd generation sequencing technologies should facilitate the development of efficacious vaccines against a broad spectrum of pathogens that cause bovine mastitis.

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